Wind and insect pollination (ambophily) of Mallotus spp. (Euphorbiaceae) in tropical and temperate forests

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Wind and insect pollination (ambophily) of *Mallotus* spp. (Euphorbiaceae) in tropical and temperate forests

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Relatively few flowering plants show ambophily (pollination by both wind and insects), and whether and when ambophily is advantageous has not been studied well. In the present study, we report ambophily in two dioecious pioneer tree species, *Mallotus japonicus* Müll.Arg. in a temperate forest of Japan, and *Mallotus wrayi* King ex Hook.f. in a tropical forest of Borneo, and discuss the conditions that contribute to the maintenance of ambophily. Both species are pollinated by wind because they set fruits even when flower visitors were excluded and because substantial amounts of airborne pollen reached female trees. Insects may also contribute to fruit set, because insects with body pollen visited female inflorescences. Because *M. japonicus* and *M. wrayi* exhibit floral characteristics that are adapted to both wind and insect pollination, ambophily may be actively maintained in the two species at the study sites and perhaps elsewhere. Whereas previous studies have indicated that ambophily is advantageous for pioneer plants because of changing wind conditions during forest succession, our preliminary data suggest that changes in population density also contribute to the maintenance of ambophily in *M. japonicus*.

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Only limited numbers of plant species are pollinated by both wind and insects (ambophily), and whether and when ambophily is advantageous has not been studied well. The present study revealed that the pioneer plants *Mallotus japonicus* and *M. wrayi* are both ambophilous. Floral characteristics adapted to both wind and insect pollination indicated that ambophily is maintained in the two species for adaptive reasons. We also discuss potential factors related to the maintenance of ambophily in these two pioneer species.

Introduction

To transfer pollen grains efficiently from anthers to stigmas, flowering plants have more or less specialised their flowers and/or inflorescences to their pollen vectors (Faegri and van der Pijl 1979). For example, animal-pollinated (mostly insect-pollinated) flowers are often conspicuous in colour and shape. In addition, they often have adhesive pollen grains and rewards for pollinators such as nectar. Wind-pollinated plants usually produce plenty of powdery pollen and inconspicuous small flowers without nectar. Their stamens and pistils are often exposed outside of the leaf-mass (Faegri and van der Pijl 1979). Possessing flowers that are suitable for both insect and wind pollination may be costly, because insect- and wind-pollinated plants are expected to allocate resources in different ways; animal-pollinated plants often possess conspicuous petals and/or smell to attract many

pollinators, whereas wind-pollinated plants produce large amount of pollen because male reproductive success depends on the number of pollen grains (Faegri and van der Pijl 1979). In addition, flower characteristics that are suitable for one pollination system often conflict with those for other systems. For example, sticky pollen grains and/or pollinia of many animal-pollinated flowers can be expected to be less likely to be delivered by wind. Only a limited number of plant species are known to employ both wind and insect pollination (ambophily).

Although ambophily is often considered an intermediate condition during a transition to either full wind pollination or biotic pollination (Culley *et al.* 2002), some studies have suggested that ambophily can be advantageous in environments where conditions favouring either wind or biotic pollination vary spatially and temporally. For example, in alpine regions, populations of effective insect pollinators decline with increased elevation (Warren *et al.* 1988), whereas wind conditions may be similar along elevational gradients (Gómez and Zamora 1996). To ensure seed production throughout an elevation gradient, having a wind-pollination system as reproductive insurance may be advantageous for some alpine plants, such as *Hormathophylla spinosa* (Brassicaceae) (Gómez and Zamora 1996) and some alpine *Salix* species (Salicaeae) (Peeters and Totland 1999; Totland and Sottocornola 2001). Other ambophilous plants are pioneer plants adapted to early stages of forest succession; they include *Salix* spp. (Salicaceae) (Tamura and Kudo 2000; Karrenberg *et al.* 2002) and *Azadirachta indica* (Meliaceae) (Vikas and Tandon 2011). Early successional forests are exposed to the wind; thus, wind pollination is suitable for such habitats (Stellman 1984; Goodwillie 1999). However, along with succession, forests gradually become enclosed and wind may diminish within the forests. Reproductive success by wind pollination may decrease, and insect pollination may become relatively more important (Stellman 1984; Goodwillie 1999).

In the present study, we examined the pollination system of *Mallotus japonicus* (Euphorbiaceae) in temperate Japan and *M. wrayi* on Borneo Island, Malaysia. *Mallotus* is a genus of ~150 species of dioecious trees or shrubs distributed mainly in palaeotropical regions. Most *Mallotus* species are pioneers, but they occur in various habitats, from secondary forests and riverbanks to the understorey of primary forests (Slik 2005; Sierra *et al.* 2007). The physical appearance of inflorescences and flowers of most *Mallotus* species appears to indicate that they are wind-pollinated; the flowers are apetalous and the anthers and stigmas are exposed. However, several studies have reported visitation by insects, such as bees and syrphids, to the male inflorescences of *Mallotus* (Lock and Hall 1982; Momose *et al.* 1998; Sierra *et al.* 2007), and our preliminary study revealed that male flowers of *M. japonicus* and *M. wrayi* produce nectar and that male and female inflorescences of *M. japonicus* have a sweet scent (E. Yamasaki, unpubl. data). The goals of the present study were to test whether wind and insect visitors contribute to the pollination of the two species and to determine which factors are involved in the maintenance of the pollination system.

Materials and methods

Study species

Trees of *M. japonicus* are distributed in temperate and subtropical regions of eastern Asia. They are dioecious pioneer trees occurring mostly in young secondary forests (Horikawa 1972). These trees grow up to 10 m in height and become reproductive from ~1 m tall and 2 cm diameter at breast height (DBH). Male trees have several-branched panicles 10–20 cm long (Fig. 1*a*). They are formed by tiny apetalous flowers that harbour 60–90

stamens (Sierra *et al.* 2010). Male flowers secrete flower nectar (0.27 µL per flower with 29% sugar content on average, as determined using a sugar refractometer to assay nectar collected by 0.5-µL microcapillaries; E. Yamasaki, unpubl. data). The pollen grains are dry and measure ~23.0 × 25.3 µm in size (Nowicke and Takahashi 2002). Female inflorescences are composed of non- or several-branched panicles 5–10 cm long formed by tiny apetalous flowers (Fig. 1*b*). Each flower has three- or four-branched dry and papillose stigmas. Female flowers do not secrete nectar. Both male and female inflorescences emit similar sweet scents. Flowering occurs almost synchronously within a population and lasts for ~2 weeks. Female flowers open synchronously within an inflorescence, whereas male flowers open sequentially and fall 1–2 days after opening. The fruits mature ~1 month after flowering. Each fruit has three or four locules.

Mallotus wrayi trees are small, up 23 m in height, distributed in Peninsular Malaysia, Sumatra and Borneo (Van Welzen and Sierra 2006). They are dioecious trees found widely in primary and secondary forests on the islands. They are reproductive from ~1 m tall and 1 cm DBH. Both staminate and pistillate inflorescences (Fig. 1*c*, *d*) are 5–10 cm long and are rarely branched. Male flowers are apetalous and have 18–40 stamens (Sierra *et al.* 2010), and the pollen grains are similar to those of *M. japonicus*. They secrete small amounts of nectar (0.04 µL per flower, with 8.6% sugar content on average, E. Yamasaki, unpubl. data). Each female flower has one pistil with a three- or four-branched dry and plumose stigma, and does not secrete nectar. We were unable to detect scent from the flowers. Each fruit has three locules. The durations of flowering and fruiting are similar to those of *M. japonicus*.

Study sites

Studies on *M. japonicus* were conducted in June and July 2009, in Seta Park, Otsu, Shiga Prefecture, Japan (34°50′N, 135°50′E). This city park is mostly covered by a young secondary forest. The study area was a bank of a small straight stream (~3 m in width). Wild pioneer plants such as *M. japonicus* and locust trees (*Robinia pseudoacacia*, Fabaceae) stand linearly along the stream banks. Annual mean temperature is 14.9°C, and mean temperatures in June and July were 21.9°C and 25.8°C, respectively (Japan Meteorological Agency, http://www.jma.go.jp/jma/index.html; 27 November, 2012). *M. japonicus* flowers from June to July at the site. Annual total rainfall is ~1500 mm (Japan Meteorological Agency).

Studies on *M. wrayi* were conducted in October and November 2009, in Lambir Hills National Park, Sarawak, Malaysia (4°20′N, 113°50′E). Temperature exhibited little annual variation, and daily maximum temperature was 32°C (Davies and Ashton 1999). Annual total rainfall is ~3000 mm (Roubik *et al.* 2005). Seasonal changes in rainfall are small, but the area irregularly experiences short-term droughts. Such droughts trigger general flowering, during which various tree species flower synchronously (Sakai *et al.* 2006). The study period coincided with the general flowering season. The area is covered by primary lowland mixed dipterocarp forest, in which trees of *M. wrayi* occur at a relatively low density.

Pollination experiment

We selected five female trees of *M. japonicus* (tagged J1, J2, J3, J4 and J5) at different distances from the nearest males (6, 12, 46, 97 and 101 m, respectively). The female trees were more than 2 m tall and 5 cm DBH, and all were mature. We conducted the following five treatments on each tree: (1) control – three to five inflorescences were tagged and left untouched; (2) insect exclusion – three to five inflorescences were covered with a fine net

(80-μm mesh, Cloth Cabin, Suminoe Teijin Techno, Osaka, Japan), which allowed pollen grains of *Mallotus*, but not insects, to pass; (3) bagged – three inflorescences were covered with paper bags (Grape Bag, DAIICHI VINYL, Fukui, Japan) through which neither pollen nor insects could pass; (4) bagged and hand-pollinated – three inflorescences were covered with paper bags and hand-pollinated while flowering; and (5) pollen supplementation – three to five open inflorescences were hand-pollinated while flowering. We placed bags or nets on the inflorescences for Treatments 2–4 on 11–13 June, and counted the number of flowers for these treatments on 2–4 July. Because all flowers opened almost synchronously, all of the studied inflorescences had not been pollinated before our treatments, and all treated flowers and inflorescences were comparable. No insects were seen on inflorescences when inflorescences were bagged. We counted the number of fruits on the inflorescences on 28 July, when the fruits were still green but fully plump. Fruit set of each inflorescence was calculated by dividing the number of fruits by the number of flowers.

For *M. wrayi*, we selected two reproductive female trees, W1 and W2, for the experiments. Both trees were more than 5 m tall, and DBH was more than 7 cm. We conducted the following treatments on each tree: (1) control – three inflorescences on W1 and 12 on W2 were tagged and left untouched; and (2) insect exclusion – three inflorescences on W1 and 12 on W2 were covered with a fine net before anthesis on 27 September. Since almost all the inflorescences flowered synchronously, all of the studied inflorescences had not been pollinated before our treatments. We counted the number of flowers on each inflorescence on 4 October, and the number of fruits on inflorescences on 2 November. Fruit set was calculated using the same procedure as for *M. japonicus*.

The effects of distance from the nearest male on the fruit set in *M. japonicus* were examined using a generalised linear mixed model (GLMM, function lmer in library lme4) in R 2.14.0 (R Development Core Team 2010). Because the dependent variables of the two models below were fruit set represented as proportion data, binomial error distribution and logit-link function were chosen. In the first model, the dependent variable was fruit set of the control inflorescences. Distance from the nearest male was included as a fixed term, and the tree individual was modelled as a random effect. In the second model examining effects on pollen limitation, fruit set of inflorescences under the control and pollen-supplementation treatments was the dependent variable. Treatments (control and pollen supplementations) and interactions between treatment and distance as well as the distance to the nearest male were included as fixed effects.

Pollen limitation of individual trees was examined by comparing fruit set of control and pollen-supplementation inflorescences for each tree by using a generalised linear model (GLM) with a binomial error distribution and logit-link function. In this model, fruit set of control and pollen-supplementation inflorescences was included as a dependent variable, and treatment (control and pollen supplementations) was a fixed term.

Monitoring of airborne pollen

For *M. japonicus*, we placed five glass slides (2.6 × 7.6 cm, Micro Slide Glass, Matsunami Glass Industry, Osaka, Japan) layered with petrolatum for 72 h (from 24 to 27 June) on the crown of each of the five female trees used for the pollination experiment. The glass slides were changed every second day. After removal, the number of pollen grains on the glass slides was counted under an optical microscope to calculate the number of pollen grains captured on the slide each day. We distinguished the pollen grains of *Mallotus* from those of other species by their size, colour, ellipsoidal shape and smooth surface.

For *M. wrayi*, we placed five glass slides layered with petrolatum for 42 h (from 30 September to 2 October 2009) on tree W2, and on two additional female trees, W3 and W4. W2 was located near a male tree (distance between the stems <2 m), and W3 and W4 were located more than 50 m from male trees. The density of airborne pollen was calculated using the same procedure as for *M. japonicus*.

To test whether the number of airborne pollen grains decreases with distance, we fitted a GLMM with a Poisson error distribution and log-link function. In the model, the number of pollen grains caught on a glass slide on 1 day was the dependent variable. Distance from the nearest male was included as a fixed term, and the date when the glass slides were set out was a random effect.

Collection of flower visitors

To investigate whether insects contribute to pollination, we captured visitors to flowers and investigated their body pollen. We captured relatively large flower visitors (mostly dipterans and hymenopterans) with insect nets. For the five female *M. japonicus* trees (J1–J5), 2 h were spent capturing visitors to each tree with insect nets. Visitors to three male *M. japonicus* trees were captured with insect nets during a total of 4 h. We were able to reach 20–30 inflorescences on each tree. Small insects that stayed on flowers (mostly hemipterans and thysanopterans) were captured using aspirators and by sampling inflorescences. At each of the five female trees (J1–J5), 1 h was spent using aspirators to capture insects that stayed on flowers. Five inflorescences from each of the five female trees (J1–J5) and one inflorescence from each of three male trees were sampled, and all insects found on the inflorescences were kept.

For *M. wrayi*, 3 h were spent at each of three female trees and 1 h was spent at each of three male trees to capture flower visitors with insect nets. Although the trees were more than 5 m tall, inflorescences were observed from ~1.5 m, and we were able to reach 10–30 inflorescences on each tree. To capture small insects, 7–26 inflorescences from each of six female trees and three inflorescences from each of three male trees of *M. wrayi* were sampled.

Captured insects were identified to the order level, except for Hymenoptera, which was classified to superfamily. The body pollen of insects captured on female trees was quantified under a stereomicroscope. We investigated whether visitation frequency of each of the six insect orders (see Results) was correlated with the distance from the nearest male by using Spearman's rank correlation tests.

Results

Fruit set

For *M. japonicus*, fruit set of the control inflorescences was 59.7–93.5% and did not significantly change with distance from the nearest male (Fig. 2, GLMM, $\chi^2 = 0.19$, $P = 0.66$). When insects' access to flowers was excluded by a net, all inflorescences set fruits, although the proportion was much lower than for control inflorescences (Fig. 2, 14.8–68.4%). In contrast, none of the flowers under the bagged treatment set fruit, whereas the bagged inflorescences with supplemental hand-pollination showed 61.5–100% fruit set. GLMM analysis on fruit set of inflorescences under the control and pollen-supplementation treatments showed that the interaction between the treatment and the distance from the nearest male was a highly significant predictor of seed set ($\chi^2 = 34.75$, $P < 10^{-8}$, Fig. 2) as was the effect of treatment ($\chi^2 = 5.87$, $P = 0.02$). Fruit set significantly differed between control and

pollen-supplementation inflorescences in Tree J2 (GLM, $\chi^2 = 5.01$; $P = 0.03$) and particularly in Trees J3, J4 and J5 ($\chi^2 = 55.73, 66.48, 55.28$; $P < 10^{-12}$) but not in J1 ($\chi^2 = 0.02$; $P = 0.88$).

On *M. wrayi*, fruit set of open and netted inflorescences was 0–58.3% and 0–33.3%, respectively (Fig. 2).

Airborne pollen

Substantial amounts of pollen of *M. japonicus* and *M. wrayi* were captured by glass slides on all female trees investigated in both species; 138.9 ± 96.1, 79.3 ± 70.8, 21.1 ± 16.5, 13.1 ± 11.2 and 14.3 ± 10.3 pollen grains of *M. japonicus* reached J1, J2, J3, J4 and J5, respectively, per slide per day, and 26.3 ± 27.3, 6.1 ± 4.1 and 0.8 ± 1.3 pollen grains of *M. wrayi* reached W2, W3 and W4, respectively, per slide per day. The amount of airborne pollen of *M. japonicus* considerably decreased with distance from a male tree (GLMM, estimated coefficient of distance = –0.03, $\chi^2 = 1842.1$, $P < 10^{-15}$).

Flower visitors

In total, 100 and 111 flower visitors belonging to various orders were collected from female trees of *M. japonicus* and *M. wrayi*, respectively (Table 1). Female inflorescences were visited less often by insects than were male inflorescences. The most frequently captured flower visitors during inflorescence collections were thrips (Thysanoptera) on both males and females. These insects stayed on the inflorescences, stuck their proboscises into the filaments or the stigma and sucked the juice. Few of the thysanopterans captured on female inflorescences carried any pollen on their bodies (18% on *M. japonicus* and 1% on *M. wrayi*; Table 2). The most frequently captured flower visitors during insect-net collections on female inflorescences of both tree species were hymenopterans. Among these, most Vespoidea (family Vespidae) on *M. japonicus* (100%) and Apoidea on *M. japonicus* (family Apidae, Halictidae and Andrenidae) (67%) and *M. wrayi* (*Apis dorsata*, Apidae) (100%) had large pollen loads (>11 pollen grains; Table 2), especially on their heads and legs. These insects stayed only for a few seconds on the female inflorescence, whereas they collected both nectar and pollen on males. Some of the other visitors (dipterans, hemipterans, coleopterans and lepidopterans) also had high or low numbers of pollen grains (Table 2). The number of insect visitors and distance from the nearest male tree were not significantly correlated on females of *M. japonicus* (Spearman's rank correlation test, $P = 0.08$–0.56).

Discussion

The results of the present study suggest that *Mallotus japonicus* and *M. wrayi* are both wind- and insect-pollinated (ambophilous). Both species are wind-pollinated because inflorescences covered by nets set fruits even though all insect visitors were excluded. However, the relative contribution of wind pollination cannot be directly estimated from our results, given the possibility that a portion of airborne pollen was excluded by the extremely small mesh size of the nets. Because inflorescences covered by paper bags did not set fruit, but did when hand-pollinated, these trees do not set fruits by apomixis. The substantial amount of airborne pollen caught on all study trees also supports the effectiveness of wind pollination. In a preliminary experiment using *M. japonicus* during a previous year, all netted inflorescences also set fruits (E. Yamasaki, unpubl. data). Possible adaptations for wind pollination include the papillose and plumose stigma, the large amount of dry pollen grains, exposed anthers and stigma and elongated inflorescences of the two species. These species also appear to be insect-pollinated because insects with pollen on their bodies visited female flowers. Because most of the observed body pollen was attached to the heads and legs of

flower visitors and these body parts frequently touch the stigma when they land on inflorescences, these insects may be effective pollinators. Male inflorescences of *M. japonicus* and *M. wrayi* attracted insects by nectar and pollen. Male and female inflorescences of *M. japonicus* emitted similar odours and were similar in appearance. Male and female inflorescences of *M. wrayi* are also similar in appearance, although the odour was not as strong as in *M. wrayi*. These characteristics may represent adaptations for insect pollination. The visitation of insects to male inflorescences of *M. japonicus* and *M. wrayi* may also facilitate wind pollination by scattering pollen grains into the air, as reported for other plant species such as *M. oppositifolius*, *Cravata adansonii* and *Chamaedrea pinnatifrons* (Lock and Hall 1982; Listabarth 1993; Mangla and Tandon 2011).

For both species, the most important pollinator insects appeared to be hymenopterans such as Vespidae, Apidae, Halictidae and Andrenidae, because the visitation rates of these insects were relatively high among all insects captured by insect nets; furthermore, these insects carried high numbers of pollen grains. Hymenopterans travel relatively long distances for foraging (Proctor *et al.* 1996). In the case of *M. wrayi*, however, whether giant honeybees (*Apis dorsata*) are frequent visitors during every flowering event remains unclear. Because the abundance of giant honeybees increases during the general flowering season at Lambir Hills National Park (Itioka *et al.* 2001), the abundance and composition of flower visitors may differ when *M. wrayi* flowers during non-general flowering periods. For *M. japonicus*, we conducted flower-visitor collections for two flowering seasons in Seta Park and for one season in each of two other sites in temperate and subtropical areas of Japan (Yasu, Shiga Prefecture, and Okinawa Island). Hymenopterans were always frequent visitors (E. Yamasaki, unpubl. data). Many thrips were also observed on *M. japonicus* and *M. wrayi*, but they may contribute little to pollination, as their pollen load and visitation frequency to female inflorescences were very low. Some species of *Macaranga*, the genus most closely related to *Mallotus* (Kulju *et al.* 2007), are exclusively pollinated by thrips (Moog *et al.* 2002; Fiala *et al.* 2011), but this is not the case in the two study species of *Mallotus*.

Insect pollinators visited not only male, but also female inflorescences, even though female inflorescences did not possess any rewards such as nectar or pollen; these insects may have been deceived by the smell and/or appearance of female inflorescences similar to those of males. The African species *M. oppositifolius* may also be pollinated by various bees and flies that are deceived by smell and appearance (Lock and Hall 1982). This type of insect pollination might occur broadly in *Mallotus*. Although insect visitation to female inflorescences has not been confirmed, visitation of bees and flies has been reported for *M. griffithianus*, *M. penangensis*, *M. brevipetiolatus* and *M. paniculatus* (Momose *et al.* 1998; Corlett 2004; Sierra *et al.* 2007).

Given that floral characteristics adapted for both wind and insect pollination can be recognised in both species, ambophily in *M. japonicus* and *M. wrayi* may be actively maintained because of several advantages of this pollination system, in contrast to either accidental maintenance or a possible transitional state of the two species. In some pioneer plants, ambophily is considered a strategy to accommodate changing wind conditions during different stages of forest succession (Stellman 1984; Goodwillie 1999). In addition, we propose that changes in population density also contribute to the maintenance of ambophily. Population densities of pioneer plants such as *Mallotus* species change as forest succession progresses; densities are high in early successional forests and gradually decrease as late successional plants colonise the forests (Pacala 1996; Guariguata and Ostertag 2001). Several studies have reported that in wind-pollinated plants, pollen limitation increases rapidly with increases in

distance from a pollen source (Levin and Kerster 1974; Steven and Waller 2007; Vandepitte *et al.* 2009; Hesse and Pannell 2011). In *M. japonicus*, we also found that the amount of airborne pollen rapidly decreased with distance from the pollen source. We observed pollen limitation only in trees without males in their vicinity, which may be attributable to short-distance pollination by wind. Interestingly, fruit set of control inflorescences itself did not change with distance. One possible explanation may be varying resource availability for fruit production among trees; female trees far from males might have suffered from pollen limitation in previous years and accumulated more resources, thus setting more fruits when pollen was supplemented. In contrast, pollen limitation does not strongly depend on distance from a pollen source in insect-pollinated plants (Schulke and Waser 2001; de Jong *et al.* 2005; Albrecht *et al.* 2009). In *M. japonicus*, insects with ample body pollen, primarily hymenopterans, visited the inflorescences regardless of distance from a pollen source.

Although the data presented in the present study are still preliminary, our results may indicate that the effectiveness of wind and insect pollination may differentially depend on population density, which has rarely been examined in ambophilous plants. Ambophily has been documented only in ~10 genera, most of which were thought to be either wind- or insect-pollinated before close investigation (Culley *et al.* 2002). Ambophily may thus be more common than currently thought (Culley *et al.* 2002). Further studies may reveal that ambophily is an important mechanism to ensure reproduction for plants experiencing unstable habitats.

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Table 1. Visitation frequency of flower visitors on *Mallotus japonicus* and *Mallotus wrayi*

The data are divided by the three collection methods. Relatively large flying insects were collected using insect nets, and small insects were collected using aspirators or by sampling entire inflorescences. Means ± standard deviation (variation among trees) are shown. Various types of insects visited male and female inflorescences of *M. japonicus* and *M. wrayi*

Collection method	Taxon of insects	*M. japonicus*		*M. wrayi*	
		Female	Male	Female	Male
Insect net (per tree per h)	Hymenoptera				
	Vespoidea	0.8 ± 0.6	1.8 ± 1.9	0.1 ± 0.2	0.3 ± 0.6
	Apoidea	0.6 ± 0.9	3.3 ± 1.3	0.3 ± 0.0	16.6 ± 9.7
	Chalcididea	0.1 ± 0.2	0.3 ± 0.6	0.0	0.0
	Tenthredinoidea	0.0	0.0	0.0	0.0
	Ichneumonidea	0.2 ± 0.4	0.4 ± 0.8	0.0	0.3 ± 0.6
	Diptera	0.8 ± 0.6	1.8 ± 1.6	0.0	0.3 ± 0.6
	Lepidoptera	0.1 ± 0.2	0.0	0.0	0.0
	Number of trees	5	3	3	3
Total collection time (h)		10	4	9	3
Aspirator (per tree per h)	Thysanoptera	8.4 ± 4.0	–	–	–

	Diptera	1.0 ± 1.2	–	–	–
	Hemiptera	0.8 ± 0.8	–	–	–
	Coleoptera	0.2 ± 0.4	–	–	–
	Number of trees	5			
Total collection time (h)		5			
Sampling of whole inflorescences (per inflorescence)	Thysanoptera	1.0 ± 0.5	18.3 ± 9.0	1.0 ± 1.1	7.7 ± 3.2
	Diptera	0.1 ± 0.1	0.3 ± 0.6	0.0	0.0
	Hemiptera	0.1 ± 0.3	8.0 ± 5.0	0.3 ± 0.2	0.2 ± 0.4
	Coleoptera	0.2 ± 0.4	0.0	0.0	0.0
	Number of trees	5	3	6	3
Total number of collected inflorescences		25	3	87	9

Table 2. Proportion of flower visitors collected on female inflorescences with no (0 pollen grains, –), small (1–10 pollen grains, +) or large (>11 pollen grains, ++) pollen load

Proportions are shown horizontally by insect order. Insects captured using different methods are pooled in the table. Many insects with body pollen visited female inflorescences of *Mallotus japonicus* and *Mallotus wrayi*

Taxon	*M. japonicus*				*M. wrayi*			
	–	+	++	*N*	–	+	++	*N*
Thysanoptera	0.82	0.18	0.00	57	0.99	0.01	0.00	73
Hymenoptera								
Vespoidea	0.00	0.00	1.00	8	0.00	1.00	0.00	1
Apoidea	0.00	0.33	0.67	6	0.00	0.00	1.00	3
Chalcididea	0.00	0.00	1.00	1	–	–	–	0
Ichneumonidea	0.00	1.00	0.00	3	–	–	–	0
Diptera	0.27	0.55	0.18	11	–	–	–	0
Hemiptera	0.50	0.38	0.13	8	0.78	0.22	0.00	32
Coleoptera	0.00	1.00	0.00	5	1.00	0.00	0.00	2
Lepidoptera	0.00	1.00	0.00	1	–	–	–	0

Fig. 1. (*a*) A male inflorescence of *Mallotus japonicus*. (*b*) A female inflorescence of *M. japonicus*. (*c*) A male inflorescence of *M. wrayi*. (*d*) A female inflorescence of *M. wrayi*. Scale bars = 3 cm (*a–c*) and 1 cm (*d*).

Fig. 2. Fruit set (number of fruits per number of flowers) of *Mallotus japonicus* and *M. wrayi*. Columns show control inflorescences, inflorescences covered by nets, and pollen-supplemented inflorescences as indicated. Vertical bars represent standard deviation. Labels are the IDs of female trees.

Fig. 1

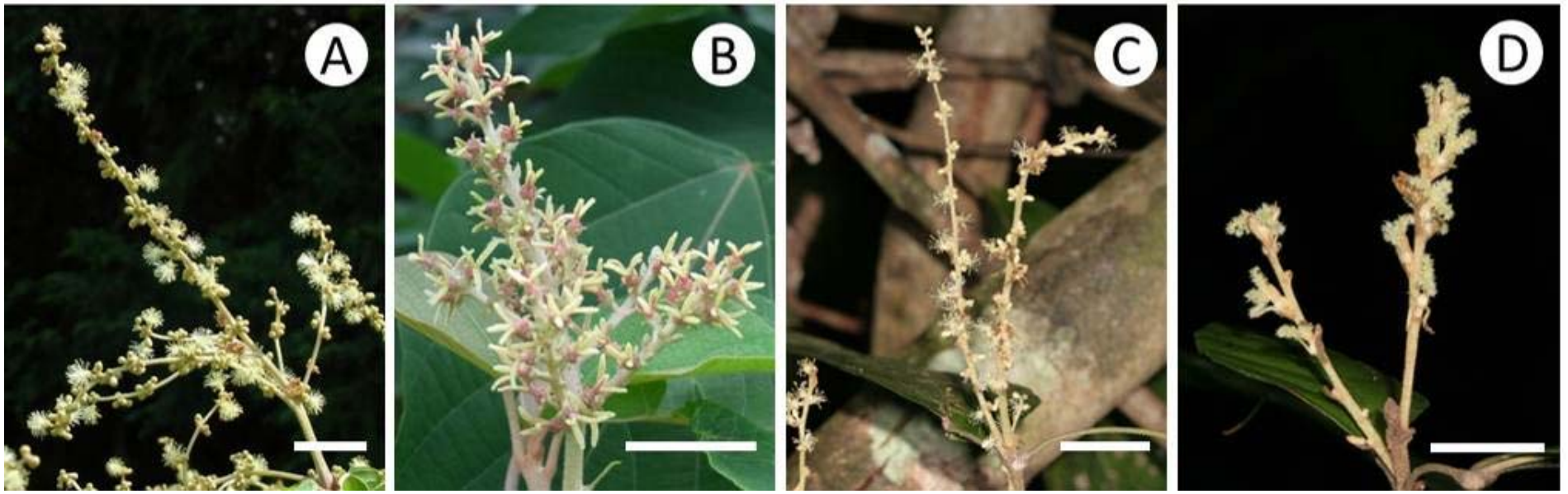
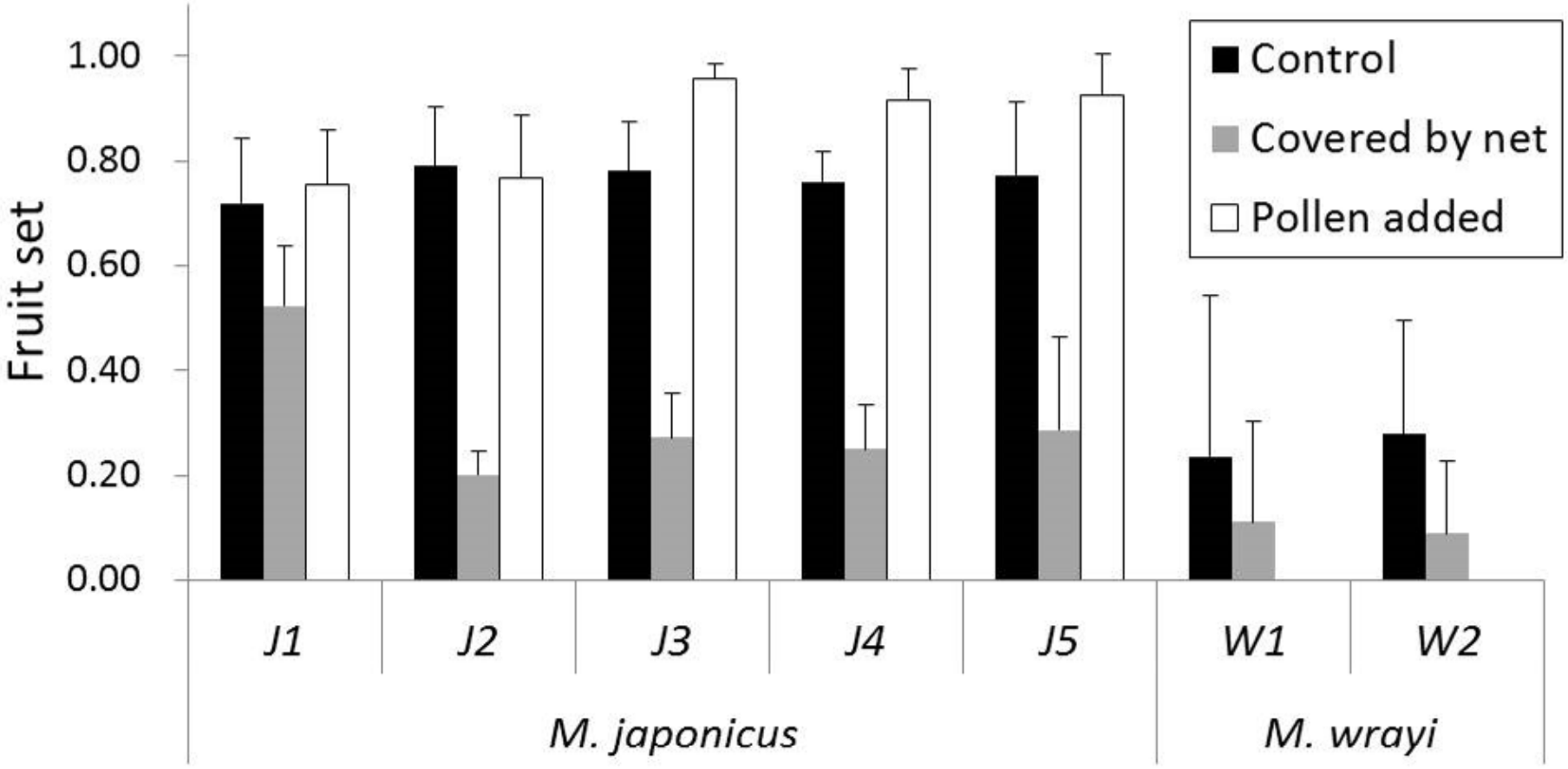


Fig. 2


Fruit set
1.00
0.80
0.60
0.40
0.20
0.00
Control
Covered by net
Pollen added
J1
J2
J3
J4
J5
W1
W2
M. japonicus
M. wrayi